Karyotypic Study of Brunneus Titi in Northern Bolivia

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ABSTRACT

A karyotypic study on a subspecies of dusky titi, *Callicebus moloch brunneus* has been carried out and the third karyotype of *C. moloch* has been discovered. The chromosome number of this subspecies is 48. The autosomes consist of 5 subtelocentric, 5 submeta- or metacentric, and 13 acrocentric chromosome pairs. The X chromosome and the Y chromosome are submetacentric and metacentric, respectively.

Comparative study of *C. moloch* group (*i.e. C. m. cupreus* and *C. m. ornatus* with 2n = 46 and *C. m. donacophilus* with 2n = 50) suggests that the karyotype of *brunneus* positions intermediate between two other karyotypes of *C. moloch,* but more similar to that of 2n = 50.

INTKRODUCTION

Two karyotypes of *C. moloch* were previously reported: two subspecies, *C. m. cupreus* and *C. m. ornatus* had 46 chromosomes (EGOZCUE, 1969; BENIRSCHKE and BOGART, 1976) and *C. m. donacophilus* 50 chromosomes (DE BOER, 1974; MINEZAWA and VALDIVIA, 1984). Comparison using G- and C-band techniques revealed that the two karyotypes differed from each other by two Robertsonian rearrangements and four pericentric inversions (MINEZAWA and VALDIVIA, 1984). These two karyotypes distributed geographically northern (*C. m. ornatus*) and southern (*C. m. donacophilus*) extremes of *Callicebus*' habitat and the differences between them are rather large as a karyotypic difference between ordinary conspecific subspecies. Therefore, in a mid-area between the habitats of two *Callicebus'* karyotypic races (2n = 46 and 2n = 50), we can expect to discover a new karyotype intermediate between the two.

This paper presents G- and C-bands of the third karyotype of *Callicebus moloch* collected at Montecarlo, Pando, Bolivia where *brunneus* titi is expected to inhabit from the description of HERSHKOVITZ (1963).

MATERIALS AND METHODS

Peripheral blood samples were taken from two female and two male dusky titi, *Callicebus moloch brunneus* captured on the south bank of Rio Manuripi, within a radius of 4 km from Montecarlo, Pando, Bolivia (Fig. 1). Whole blood samples were conserved with ice for three to

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Figure 1. Map of Bolivia and the location of Montecarlo (pointed by arrow).

five days before 72 hours' culture in RPMI-1640 (containing PHA-M, FCS and antibiotics). Cells were fixed by standard method at Instituto Bioclinico Central, Santa Cruz, Bolivia and stored at -20°C in Carnoy's acetic acid-alcohol fluid. The fixed samples were processed at the Primate Research Institute, Kyoto University, Japan. The metaphase chromosome was sequentially stained for standard Giemsa, G- and C-bands with ASG (SUMNER *et al.*, 1971) and BSG (SUMNER, 1972) methods.

At least 10 metaphase karyoplates from each specimen were observed under a microscope for counting the chromosome number and recording their gross morphology. More than two G- and C-banded karyoplates of three individuals (two males and one female) were photographed, and they were compared with each other and with two other karyotypes reported previously (BENIRSCHKE and BOGART, 1976; MINEZAWA and VALDIVIA, 1984).

RESULTS

Chromosome number of all the specimens studied is 48. Their autosomes consist of 5 pairs of subtelocentric, 5 pairs of submetacentric or metacentric and 13 pairs of acrocentric chromosomes. The X-chromosome and the Y-chromosome of this karyotype are submetacentric and metacentric, respectively.

G- and C-band karyotypes are shown in Fig. 2. Centromeric C-bands are observed on all chromosomes. Telomeric C-bands are also observed on short arms of Nos. 4, 8 and 9 chromosomes and no interstitial C-band is observed. Y-chromosome thoroughly stains dark. Using G-band techniques, we can distinguish all the chromosomes. By comparing the G-banded karyotype of *brunneus* with the two other types of *Callicebus moloch* group, *i.e.* 2n = 46 (BENIRSCHKE and BOGART, 1976) and 2n = 50 (MINEXAWA and VALDIVIA, 1984), we can find that all the chromosomes of *brunneus* possess their homologues in two karyotypes reported previously (Table 1).

Table 1 Chromosome studies in *Callicebus moloch*

Species	2n	Chromosomes			
		N-A[1]	A[2]	X	Y
C. m. brunneus	48	20	26	SM	M
C. m. donacophilus	50	22	26	SM	M
C. m. ornatus & cupreus	46	20	24	SM	M

1. Non-acrocentric 2. Acrocentric

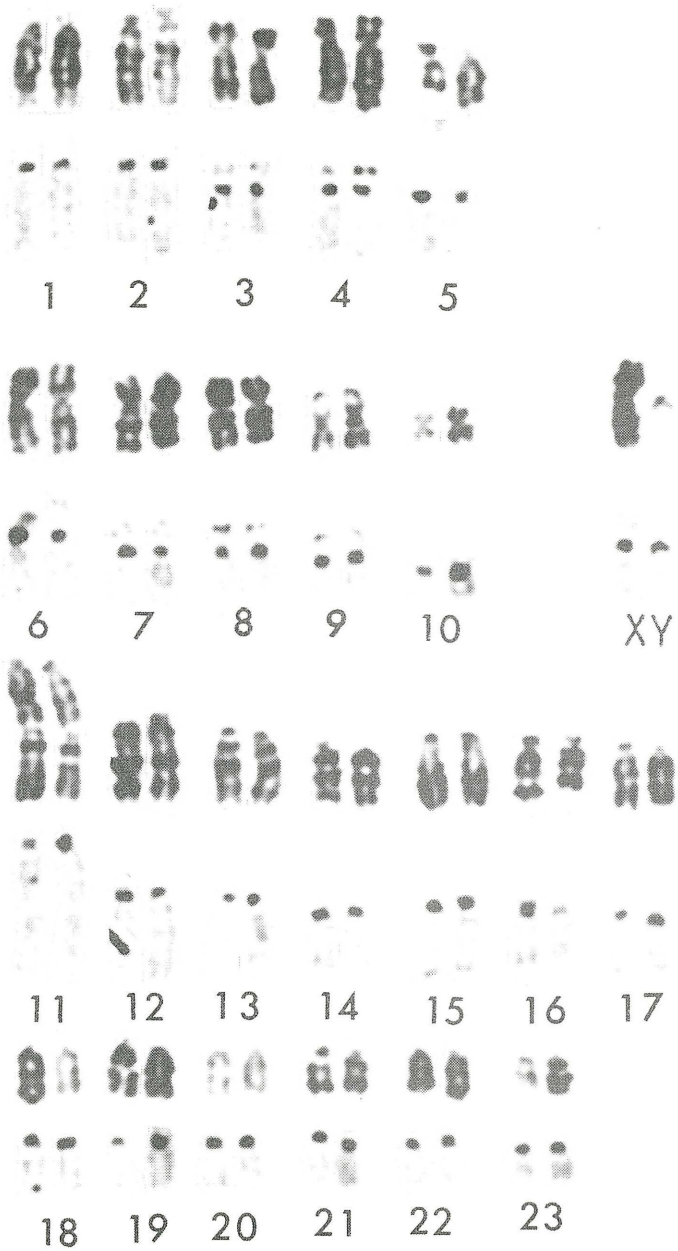

Figure 2. G- and C-band karyotype of male *Callicebus moloch brunneus.*

brunneus vs *cupreus*

The longest metacentric chromosome pair of the 2n = 46 karyotype corresponds to the longest acrocentric pair of the 2n = 48 karyotype and the difference between these two chromosomes is only a transposition of centromere or centromere shift without inversion. The second largest bi-arm chromosome pair (No. 2) of the 2n = 46 corresponds to two acrocentric chromosomes (Nos. 15 and 20) of the 2n = 48. Three acrocentric chromosomes (Nos. 12, 14 and 16) of the 2n = 46 karyotype change into three bi-arm chromosomes of 2n = 48 karyotype (Nos. 3, 4 and 5, respectively). On the contrary, one bi-arm chromosome (No. 6) of *cupreus* is the homologue of acrocentric chromosome (No. 16) of the *brunneus.*

brunneus vs *donacophilus*

The longest acrocentric chromosome (No. 11) of the 2n = 48 corresponds to two acrocentric chromosomes (Nos. 13 and 19) of the 2n = 50 karyotype. One acrocentic chromosome (No. 12) of *brunneus* changed into subtelocentric chromosome (No. 3) of *donacophilus* by a small pericentric inversion or a growth of C-negative element.

DISCUSSION

The karyotype of *brunneus* positions intermediate between Montecar *donacophilus* and *cupreus,* but nearer to that of *donacophilus* than to that of *cupreus*). From a morphological viewpoint, several taxonomists have diskcussed a relationship among these three *Callicebus moloch* titi (Table 2). CABRERA (1958) assigned *C. cupreus toppini* to a titi monkey of the area studied presently. HILL (1960) also put this animal into *C. cupreus* as *C. c. brunneus.* HERSHKOVITZ (1963) regards *brunneus* as one of the subspecies of *C. moloch* with other two subspecies discussing currently, but the key characters, which distinguich among subspecies of *C. moloch,* indicate a relatively closer relationship of *C. m. brunneus* to *C. m. cupreus* than to *C. m. donacophilus.* Whichever the differences between *donacophilus* and the other two dusky titi monkeys are at species level or subspecies level, all three taxonomists have placed *donacophilus* most remote position among those three *C. moloch* subspecies. As mentioned above, the karyotypical results are contradictory to the morphological observations. Similar contradictions between classification and karyotype are often observed among primates. For examples, many congeneric species of *Saguinus* species share almost the same karyotypes as 2n = 46 (HERSHKOVITZ, 1977). Although previously all owl monkeys classified as one species, *Aotus trivirgatus,* many largely differentiated karyotypes have been discovered one after another (BRUMBACK, 1974, 1975; REUMER and DE BOER, 1980; MA, 1981). As a result of those karyotypic findings, HERSHKOVITZ (1983) has come to split the genus *Aotus* from one species to nine species. Similarly genus *Saimiri* has come to be divided from one or two species to four species (HERSHKOVITZ, 1984). Though, all species of the genus *Saimiri* show the same chromosome number, 2n = 44, regional populations differ from each other by two pericentric inversions (JONES and MA, 1975). When considered the instances described above,

Table 2 A comparison of classifications of *Callicebus moloch*

CABRERA (1958)	HILL (1960)	HERSHKOVITZ (1963)
C. ornatus	*C. cupreus ornatuas*	*C. m. ornatus*
C. cupreus	*C. cupreus cupreus*	*C. m. cupreus*
C. cupreus toppini	*C. cupreus brunneus*	*C. m. brunneus*
C. moloch donacophilus	*C. gigot donacophilus*	*C. m. donacophilus*

the cytogenetic differences among three subspecies of *C. moloch* are large enough to split them into, at least, thee independent species. Moreover, the morphological key characters to distinguish conspecific subspecies of *Callicebus moloch* show almost the same degree of morphological difference as those of congeneric species of *Saimiri* and *Aotus* (HERSHKOVITZ, 1963, 1983, 1984). Therefore, it is necessary to reconstruct interrelationships among subspecies of *C. moloch* by cytogenetic method.

According to HERSHKOVITZ (1963), *C. moloch* may have originated in the highland of Southern Brazil. Whence it spread to the present habitat, and *C. torquatus* evolved from *C. moloch* in the area between the upper Rios Napo and Guaviare. *C. personatus* also evolved from *C. moloch* in the course of dispersal to the coastal forest of Southeastern Brazil. He also suggested from his "centripetal dispersal" view that the major Amazonian tributaries acted as barriers between populations spreading downstream along gallery forests and racial divergence increased with downstream spread. However, KINZEY (1982) showed that distribution paterns of *Callicebus* was largely the product of Pleistocene climatic fluctuations and the repeated disruption of forest and fitted in with the Haffer's model for the neotropical forest biota. The discussion on speciation process about *Callicebus,* by HERSHKOVITZ (1963) and KINZEY (1982), has been done without detailed cytogenetic informations. If data of karyotypes of the other subspecies could be available, a discussion on the way of differentiation of *Callicebus* would be more fruitful and more clear-cut.

The discovery of the third karyotype of *Callicebus moloch* in this study strongly suggests a necessity of detailed re-examinations of this species from morphological as well as genetic points of view.

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